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16. ABSTRACT

The author discusses sample preparation, separation of xenobiotic chemicals from the bulk of the environmental substrate, and problems associated with pollutant analysis. The application of mass spectrometry (MS) to the detection of chemicals foreign to biology is reviewed, and cases are cited to demonstrate the use of MS in identifying or quantifying xenobiotic chemicals in samples obtained from the food chain, from contact sources, or from studies of human tissues and biofluids.

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CHAPTER 32A

TOXIC RESIDUES AND POLLUTANTS

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A. INTRODUCTION

The application of MS to the detection of xenobiotic chemicals, chemicals that are foreign to biology, in the environment has significantly increased our understanding of environmental contamination. For example, MS investigations have clarified the analytical interferences between DDT and its metabolites and polychlorobiphenyls in environmental extracts (1); mass spectral studies first identified mirex and its precursors in samples of Lake Ontario fish (2); MS studies have increased our awareness of general contamination of the human population with pentachlorophenol (3); and MS studies were first to show that the mutagenic flame retardant tris(2,3-dibromopropyl) phosphate is absorbed through the skin of children wearing flame-retardant-treated garments (4).

The suitability of MS to studies of contaminants of the environment stems from three of its major strengths as an analytical technique. First, mass spectra give molecularly specific identification for a very wide variety of chemicals. Second, extremely complex mixtures can be handled by use of a number of selected MS techniques. These include GC/MS, high-resolution MS, negative chemical ionization (NCI) MS, and field desorption (FD) MS. Finally, modern mass spectrometers have enough sensitivity to detect environmental

contaminants at the low end of the range of ecological and biological concern (i.e., low parts-per-billion concentrations).

The dramatic increase in studies of environmental contamination within the last decade has been caused in part by the virtual epidemic of cancer and birth defects in the United States and by the substantial increase in federal funding for projects examining environmental contamination. These factors have caused a quantum upsurge in the number of manuscripts dealing with the application of MS to environmental analytical toxicology. The reviews of MS that appear in *Analytical Chemistry* routinely discuss pertinent recent studies (6). In addition to these reviews, others devoted to the application of MS determination of toxic residues and specific compounds in the environment have appeared within the last few years. Biros carefully reviewed the applications of MS and GC/MS to the analysis of pesticide residues prior to 1971 (7). Essentially the same subject plus CIMS was covered by Oswald et al. in 1974 (8). Two comprehensive reviews have appeared more recently. Alford has reviewed all aspects of the MS investigation of environmental pollution, including spark-source MS of airborne particulates, GC/MS, and other techniques (9). Ryan has treated the applications of MS to analysis of pesticidal compounds and their metabolites in environmental substrates (10).

The MS of pesticides as pure compounds is extensively reviewed in Chapter 23 of both the original volume of this book and in this supplementary one. Safe and Hutzinger have compiled a book-length collection of mass spectra of pesticides and toxic substances (11). An extensive catalog of EI spectra of chlorodioxins and related molecules appears in two volumes on dibenzodioxins and their impact on the environment (12, 13). Efforts have been made to increase the number of mass spectra of pesticides and toxic substances in the NIH/EPA mass spectral search system (MSSS) (14). This now contains a large number of EI spectra of xenobiotic chemicals and is currently acquiring both CI and NCI mass spectra.

1. Scope

In view of the number of excellent recent reviews of literature, this discussion will be selective rather than comprehensive. We will consider sample preparation, separation of xenobiotic chemicals from the bulk of the environmental substrate, and problems associated with extremely small amounts of material. We will review application of MS to pollutant analysis, and cite specific cases where MS has been used to identify or quantify xenobiotic chemicals in samples obtained from the food chain, from contact sources, or from studies of human tissues and biofluids.

2. Special Problems Associated with Pollutant Analysis

The special problems of environmental analytical toxicology may be summarized in five words: level, recovery, interference, blank, and calibration.

It is very seldom that the concentration of environmental contaminants in a real sample will exceed 1 part per million. The problems of separating 1 part per million or smaller quantities of toxic substances from an environmental matrix are central to determination of these residues. Virtually all strategies for analysis employ either an extraction or a crude separation, then usually some form of chromatography and MS analysis. The methods of choice in MS are those that permit one to deal with exceptionally complex mixtures of materials. The reason for this choice is that if recoveries are to remain high, the initial separations are condemned to be less than perfectly efficient.

The most widely used technique, GC/MS, requires knowledge of the molecular character of the compounds that one is looking for, to ensure that these compounds will be separated under the GC conditions used. Other MS methods for dealing with environmental contamination can produce relatively simple spectra for each of the components of complex mixtures: CI, NCI, FD, and mass-analyzed ion kinetic energy spectrometry (MIKES). The application of each of these techniques is discussed below.

In the area of environmental science consideration should be given to "levels of concern" as well. Use of state-of-the-art MS coupled with state-of-the-art sample preparation and concentration permits detection of 1 part in 10^{15} to 1 part in 10^{18}. The data in Table 32A-I suggest that concentrations of toxic substances in these ranges will seldom be significant in environmental toxicology. A concentration of 1 femtogram per gram of a toxic substance of molecular weight 300 daltons corresponds to one molecule in 500 cells, for average mammalian cells. This concentration must be considered insignificant, since most cells are

Table 32A-1 Numbers of Molecules per Cell (Molecular Weight 300 Daltons)

Level	Prefix	Number of Molecules/Cell
10^{-6}, ppm	Micro-	2×10^{6}
10^{-9}, ppb	Nano-	2×10^{3}
10^{-12}, ppt	Pico-	2
10^{-15}	Femto-	2×10^{-3}
10^{-18}	Atto-	2×10^{-6}

contaminated with toxic substances at the part-per-million level, which corresponds to roughly 10^6 molecules per cell!

The problem of recovery is closely related to the low concentrations of molecules that one seeks in analyzing environmental materials. Recoveries are seriously affected by such factors as adsorption, aerosol formation during evaporation, and incomplete extraction. Concentration of a solution containing a toxic substance in nanogram quantities by means of a conventional rotary evaporator will often reduce recovery to 10%. Thus the concentration steps in pollutant analysis usually employ Snyder columns and Kuderna-Danish concentrators. Adsorption effects should be minimized in the cleanup procedure. Inefficient extraction as well as other effects can be monitored by investigating recoveries for spiked samples in a realistic matrix.

The use of MS detectors minimizes interferences that have in the past plagued environmental analysts. Even with HR mass spectrometers, however, such complications can arise. In the determination of tetrachlorodibenzo-*p*-dioxins (TCDDs) in environmental samples, three interferences occur with high frequencies. The first two, which can generally be eliminated by use of HRMS, are the $(^{37}Cl)^+$ ions from DDE at m/z 320 and 322 and the $(^{35}Cl)^+$ ion in the $(M-2)^+$ cluster from pentachlorobiphenyls. Since both DDT and pentachlorobiphenyls are ubiquitous in environmental samples and often present in the part-per-million range, they can interfere significantly in analysis for TCDD using the ions at m/z 320 and 322. The third interference in TCDD determination is much more insidious. This results from the oxidation of tetrachlorobiphenyldiols to tetrachlorophenylbenzoquinones. Such oxidation occurs during the sulfuric acid step in the cleanup generally used. The diols are known metabolites of penta- and hexachlorobiphenyls and also appear as contaminants in some 2,4,5-trichlorophenoxyacetic acid herbicides. Fortunately, the tetrachlorophenylbenzoquinones are not coeluted with TCDD in GC. Unfortunately, these molecules remain in a mass spectrometer ion source for many hours after their introduction.

Keeping laboratory and sample collection and preparation systems clean to levels below 1 part per million is a strenuous requirement that faces every environmental chemist. It generally takes weeks for people new to the area to become familiar with the mass spectra of the lipids associated with fingerprints or the plasticizers that are so common in laboratory and other environments. The problem of blanks is particularly difficult with compounds that are persistent, widely distributed, and produced in high volume. This group includes pentachlorophenol, phthalate esters, and polyhalobiphenyls. It is generally necessary to use solvents distilled in glass to keep backgrounds at an acceptable level. If adsorption chromatography is part of the cleanup procedure, the adsorbents must be unusually clean. Commercial Florisil that is perfectly acceptable for preparative chromatography of organic chemicals will impart an unbelievable string of residues in a cleanup for toxic substances. Contamination from greases can also be a problem. Pure hydrocarbon stopcock grease will produce acceptable results; however, most investigators simply do not use any stopcock grease.

The final special problem associated with analysis of toxic residues is calibration. The most reliable procedure, the method of choice, is isotope dilution MS. Use of a stable-isotope spike for the contaminants of interest has three features that make it extremely attractive for calibration. First, an excess of the labeled spike provides a carrier for the trace contaminant. It is often possible to add a thousandfold excess of a spike to a sample prior to cleanup so that losses due to adsorption on chromatographic columns or other causes will be minimized. The isotope dilution standard also allows the calculation of absolute recovery in the sample. This is done by measuring relative abundances for the normal and isotopically substituted components and then recording the same spectrum with the addition of a known amount of the normal-isotope standard. Finally, possibly the major advantage of isotope dilution is the fact that the calibration curve for isotope dilution MS measurement of concentrations is a straight line. This is not necessarily true for any other calibration procedure, particularly if concentrations range over two orders of magnitude.

The second most reliable calibration procedure is the use of an internal standard that is structurally distinct from the compound of interest but has a similar vapor pressure. Calibration curves so prepared can be linear over a concentration range of two orders of magnitude if careful attention is paid to factors such as temperature, source pressure, and the time after injection that the spectrum is measured. Calibration curves for this kind of experiment should be checked regularly.

External calibration has been used by a number of investigators to determine approximate concentrations of toxic substances in environmental samples. In this procedure a calibration curve for ion abundance, on a arbitrary scale, is prepared as a function of the amount of sample introduced into the source. Ion abundances in the samples of interest are then converted into concentrations by using the calibration curve. This is considerably less reliable than the procedures mentioned above because the instrument response for a given compound may vary strongly with source condition and will virtually always do so with the matrix in which the sample is introduced.

B. SAMPLE PREPARATION

1. Lipid-Rich Samples

The Food and Drug Administration pesticide analytical manual (15) is the cornerstone of most sampling and cleanup procedures used for examining environmental samples. Prior to the general availability of MS for investigations of environmental contamination the most widely used detector was an electron capture gas chromatograph. The methods used to detect multiple toxic residues in this way were developed in large part by Mills, Olney, Gaither, and their coworkers (15, 16). The Mills procedure involves extraction, chromatography on Florisil, and electron capture GC or GC/MS.

Biros and Walker have reported a GC/MS study of multiple pesticide residues in human tissues in which the cleanup procedure was closely patterned after that by Mills (17). Five-gram samples of human fat and liver were homogenized and extracted and fractionated on pesticide-grade Florisil. The second fraction from the Florisil cleanup was hydrolyzed with sodium hydroxide to eliminate coeluted lipids. The two Florisil fractions were then further purified by chromatography on silica gel. Fractions from silica gel chromatography were concentrated and subjected to GC/MS.

Adsorption chromatography, which is central to the Mills cleanup procedure, has a number of disadvantages for studies of pollutants at residue levels. The most significant drawback is that the adsorption columns remove many polar and polarizable toxic substances from the extract. In consequence a number of new multiresidue methods have been developed within the last 2 years. The most promising new cleanup procedures are gel permeation chromatography (GPC), trapping of residues on Micro CEL-E, and steam distillation.

Gel permeation chromatography cleanup techniques have been developed by Stalling and coworkers (18) and Kuehl and Leonard (19). Gel permeation using commercially available gels such as Sephadex LH-20 or Biobeads SX-3 separates molecules according to molecular volumes in a nonadsorptive permeation process. High-molecular-weight substances characteristically are eluted in the first fractions. It happens that neutral lipids, which are the most difficult molecules to remove in the cleanup of environmental samples, have molecular weights that exceed 600, whereas most toxic substances of current environmental concern have molecular weights of half that much or less. Thus GPC can be used to separate neutral lipids from toxic substances without resorting to adsorption chromatography. Gel permeation chromatography generally gives recoveries equal to, or exceeding, those of the Mills procedure, and will pass many pollutants such as pentachlorophenol that are very difficult to elute from Florisil columns.

Rogers introduced a procedure for removing the bulk of neutral lipids from environmental samples which calls for adsorption of the lipids on Micro CEL-E, a commercial Celite preparation (20). The extract is mixed with Micro CEL-E and the resulting powder extracted with mixtures of acetone and acetonitrile; these remove the nonpolar substances (e.g., PCBs) in preference to the neutral lipids. The Micro CEL-E cleanup results in an approximately tenfold enrichment of the sample in nonpolar toxic substances with respect to the associated lipids. However, polar toxic substances such as pentachlorophenol and 2,4,5-trichlorophenoxyacetic acid are strongly adsorbed, which restricts the utility of this method for general-purpose screening.

Vieth and coworkers have combined the Micro CEL-E and gel permeation cleanup steps to pre-

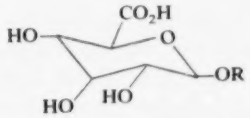
pare extracts for GC/MS (21). The high capacity of the Micro CEL-E cleanup step was used to eliminate the bulk of the lipids from fish extracts. This was followed by GPC cleanup to produce a final extract which contained sufficiently small amounts of lipids to permit examination by GC/MS. This combination of methods appears to have more efficiency and larger capacity than either the Micro CEL-E or GPC cleanup procedure by itself. The method is again limited by the fact that Micro CEL-E strongly adsorbs polar toxic substances.

One of the most intriguing new developments in cleanup methodology is the use of steam distillation by Veith and Kiwus (22). Steam distillation permits remarkable concentration of toxic substances with reference to neutral lipids and other biomolecules and recoveries appear to be excellent. Steam distillation is attractive because polarizable toxic substances are virtually all steam-volatile, whereas biomolecules which are either water-soluble or relatively nonpolarizable and of high molecular weight (e.g., lipids) do not steam-distill. When long distillation times are used, free fatty acids in the C_{16}, C_{18}, and C_{20} molecular-weight range do steam distill (23). The presence of the free fatty acids can complicate screening, particularly if the samples are introduced on a direct probe for NCI analysis (23).

Cleanup procedures that are used for detecting or determining toxic substances in lipid-free or low-lipid samples are generally less cumbersome than those for lipid-rich ones.

2. Low-Lipid Samples; Conjugates in Biofluids

Urinary excretion is one of the major export pathways for toxic substances and their metabolites in higher animals. Urine is a very convenient material to examine for residues because it contains virtually no lipids. This convenience is also somewhat of a complication because it means that all the lipophilic toxic substances have been converted into water-soluble derivatives prior to excretion. The common conjugates, water-solubilizing derivatives, for urinary excretion are glucuronic acid (**1**), taurine (**2**), and sulfate esters (**3**).



1

Glucuronide conjugate

$HO_3SCH_2CH_2NHR$

2

Taurine conjugate

$HO_3SO—R$

3

Hydrogen sulfate conjugate

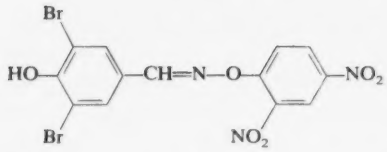
In order to analyze urine for toxic residues, other than those that are excreted in unconjugated form, one must either hydrolyze the conjugates or derivatize them so that they will have sufficient vapor pressures for MS.

Hydrolysis of urinary conjugates is accomplished by either enzymatic or acid–base routes. A mixture of glucuronidase and sulfatase effectively catalyzes hydrolysis of the conjugates of 2,3-dibromopropanol and permits analysis for this compound by NCI or negative APIMS (4). 2,3-Dibromopropanol is the major metabolite of the flame retardant tris-(2,3-dibromopropyl) phosphate. Sulfuric acid hydrolysis also liberates 2,3-dibromopropanol from conjugates, but it appears that the yields are lower when sulfuric acid hydrolysis is used (4). This decrease is probably due to dehydration and subsequent reactions of the dibromopropanol in sulfuric acid. However, sulfuric acid hydrolysis of urinary extracts at 80–100°C is effective for cleaving the conjugates of stable molecules such as pentachlorophenol and 2,4,5-T (3).

Another approach to the problem of urinary conjugates is to derivatize the conjugates themselves and measure them directly. Mrochek and Rainey have described a procedure (24) for isolating urinary glucuronides by preparative ion exchange chromatography. The glucuronides were then converted to TMS derivatives and these are analyzed by GC/MS.

For some low-lipid samples useful MS results can be obtained on only partially purified hexane extracts. Hutzinger and Jamieson have reported identifying organochlorine residues in crude extracts by MS (25). In one experiment, detection levels for the fungicide 2,6-dichloro-4-nitroaniline were of the order of 10 ppb. The procedure involved hexane extraction of peaches, drying and evaporation of the extract, and direct examination by both LRMS and HRMS.

Some compounds present particularly difficult problems in analysis regardless of the matrix in which they are contained. Karlhuber et al. reported that analysis for bromofenoxim (**4**), a herbicide that is used on hops, did not give quantitative results (26), even though the cleanup procedure involved hydrolysis, oxidation to 3,5-dibromo-4-hydroxybenzoic acid, esterification



4
Bromofenoxim

with diazomethane, and scanning only the base peak of the ester. It is conceivable that a more direct analyses of hop extracts would supply adequate sensitivity and eliminate the difficulties associated with the complex cleanup procedure.

C. MASS SPECTROMETRIC METHODOLOGY

Extracts or steam distillates of environmental samples that have been subjected to one or more cleanup steps are still complex in composition. This complexity requires that MS of toxic substances in the samples must have both high selectivity (ability to specifically identify a single compound in a mixture of hundreds of components) and high sensitivity (ability to reliably detect ~1 ng per injection or ~1 ppb). These requirements have been met by using either GC/MS in which the GC resolution has been dramatically increased with glass capillary columns, or mass spectral techniques that produce exceptionally simple spectra, such as CI, NCI, and FDMS.

1. Gas Chromatography/Mass Spectrometry

Figure 32A-1 illustrates a gas chromatogram obtained from a hexane/diethyl ether extract (of Lake Superior trout) that had been subjected to chromatography on Florisil followed by fractionation on silica gel (27). This highly fractionated sample produced the tracing of Fig. 32A-1 with a minimum of 22 components. The identities of the polychlorinated molecules corresponding to the labeled peaks in the gas chromatogram are indicated in the legend. Major contaminants (greater than 50 ppb) found in this study included polychlorobiphenyls and DDT and its metabolites. Among lower-level components were hexachlorobenzene, *cis* and *trans* isomers of chlordane and nonachlor, and molecules related to toxaphene (27).

One of the limitations of GC/MS analyses of environmental materials is the dynamic range of the entire system, including the computer. If one wants to examine the low-level components of environmental extracts, it is often necessary to drive the response for the high-level components completely off scale. This may or may not be a disadvantage, but it is intrinsic to virtually all of the analytical approaches using MS to study the environment.

Kuehl has introduced a new technique for managing the volumes of data generated in environmental GC/MS, called selected ion summation (SIS). In SIS plots, the sum of the abundances of selected masses is plotted as a function of GC/mass spectrum number. Ion abundances are included in the sums if and only if all such masses are present at preset threshold values. Figure 32A-2 (57b) presents a comparison of an ordinary mass chromatogram and a SIS trace for mirex and photomirex in Lake Ontario fish. This figure shows the unique ability of SIS to easily identify minor components of complex mixtures. This technique, along with GPC (19), has permitted the isolation and identification of polar molecules such as chlorobromophenols, bromoanisoles, and bromoindoles from fish tissue at the 1 to 5-ppb level (57).

The use of GC/low-resolution MS for the quantitation of nitrosamines in foods has been criticized because it produced results seriously different than other methods of nitrosamine determination (28). However, GC coupled with high-resolution MS gave results that were consistent with those of infrared chemiluminescence measurement of nitrosamine levels (29).

2. High-Resolution Mass Analysis

High-resolution MS offers a substantial increase in selectivity as compared to GC. The failure of GC/low-resolution MS to give reliable quantitative data on nitrosamines (28) can be traced directly to the presence of isobaric ions that appear in the ion source as a result of ineffective GC separation. High-resolution MS has been successfully used to separate ions derived from the compounds of interest from those of unit mass isobars that are difficult to remove during the introduction of the samples.

High-resolution mass analysis in GC/MS was first applied to the estimation of alkylnitrosamines by Crathorne et al. (29). These authors used isomers of the carcinogenic nitrosamines to locate the precise mass of the ion of interest that would occur in GC/MS analysis.

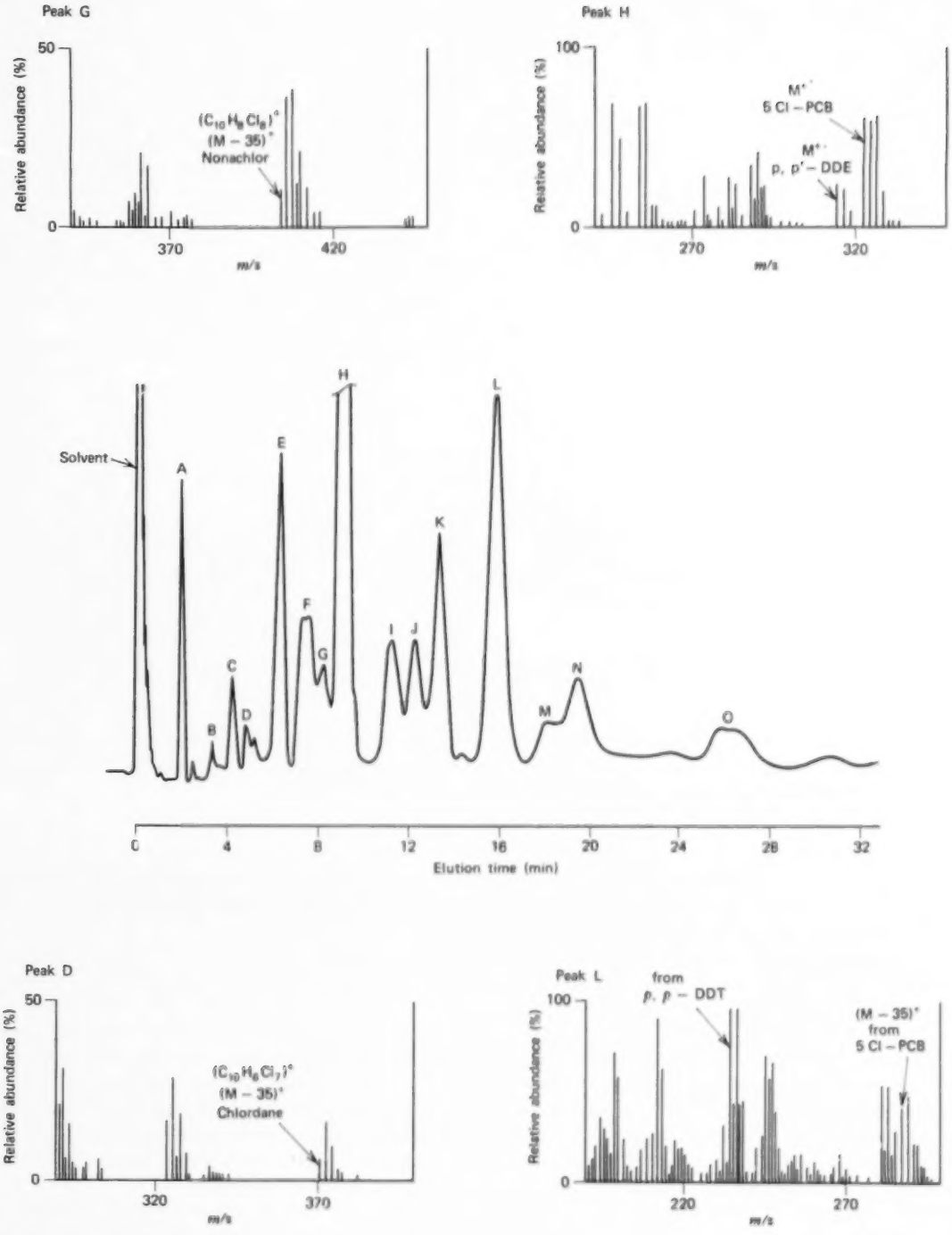


Figure 32A-1. Gas chromatogram of a Lake Superior trout extract and mass spectra of selected peaks of the chromatogram. On the basis of GC retention time and GC/MS analysis, peak A represents hexachlorobenzene (C_6Cl_6); peak D, chlordane; peaks F and G correspond to nonachlor isomers; peak H represents *p,p'*-DDE; peak I, *o,p'*-DDT; peak K, *o,p'*-DDE; and peak L, *p,p'*-DDT (27).

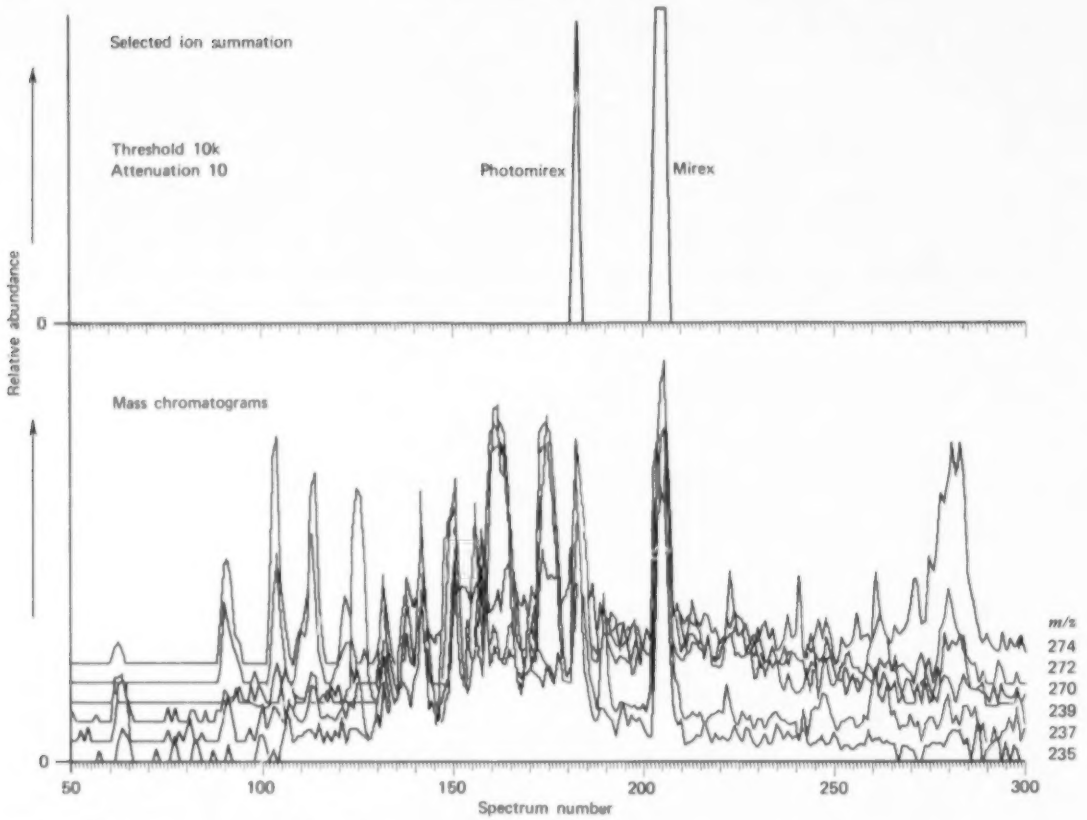


Figure 32A-2. Comparison of GC/MS data presentation for a gas chromatogram of a Lake Ontario alewife extract. Upper trace: SIS presentation requiring the presence of the $(C_5Cl_6)^+$ and $(C_5Cl_5)^+$ isotope clusters. Lower trace: conventional selected ion monitoring output (57b).

A large use of high-resolution MS in the analysis of environmental contamination was started with the work of Baughman and Meselson on determining TCDD (5). Most MS quantitation of TCDD in the environment since then has also used high-resolution MS (30–35). This is necessary because of the presence of DDE and pentachlorobiphenyls that produce isobaric ions at m/z 320 and 322, the first two ions observed in the TCDD molecular ion cluster. If the dynamic range requirements, which are determined primarily by the efficiency of the separation in the GC inlet, are sufficiently small, a resolving power of 10,000 is adequate to separate all the interfering ions (see Fig. 32A-3).

Selectivity similar to that of high-resolution MS can be obtained by the use of CI or NCI mass spectra of GC effluents for the analysis of polychlorodioxins (see below) (36).

3. Chemical Ionization and Negative Chemical Ionization Mass Spectrometry

Ion–molecule MS, CI, and NCI techniques offer a number of distinct advantages for analyzing environmentally derived mixtures. Both CI and NCI mass spectra contain relatively small numbers of peaks as compared with their EI counterparts. Sensitivities are generally high, and in NCI MS selectivity in ion formation often permits detecting low levels of toxic substances in the presence of substantially larger amounts of biomolecules such as neutral lipids.

Negative chemical ionization mass spectra of methylene chloride extracts of human urine present a wealth of information concerning the components of the extract that strongly attach anions or have high electron affinities (3). Figure 32A-4 illustrates the NCI spectrum of such a human

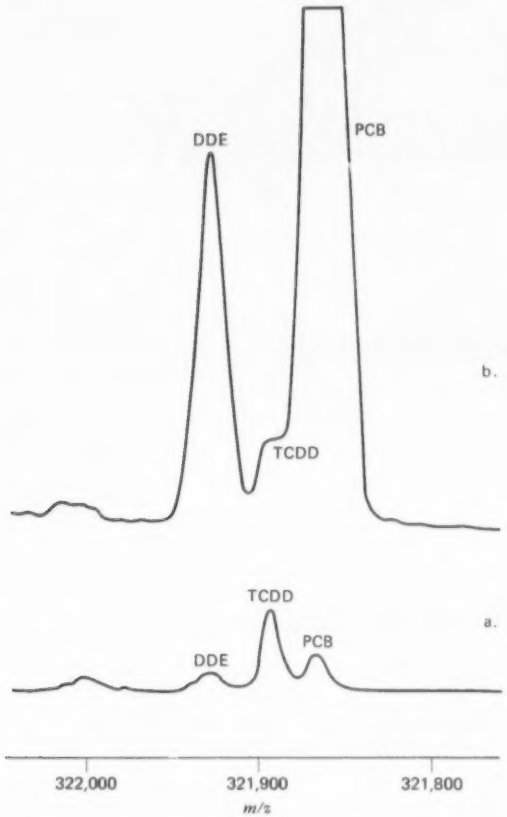


Figure 32A-3. Use of high resolving power to limit interferences from isobaric ions. These mass spectra show reduction of DDE and PCB levels in fish residue by means of alumina chromatography. Following the sulfuric acid cleanup step, the residue in hexane is added to a column of activated alumina. (*a*) Trace from the material eluted by 20% CH_2Cl_2 in hexane after the column was first washed with 20% CCl_4 in hexane. (*b*) Trace obtained from a similar 20% CH_2Cl_2-in-hexane elution after the column was first washed with 1% CH_2Cl_2 in hexane (30).

urinary extract. The spectrum clearly shows the presence of pentachlorophenol as the anion at m/z 263 and a presumably aromatic hexachloride at m/z 312 in addition to many other polyhalogenated ions. It seems very likely that a significant number of the negative mass-defect ions above m/z 350 are associated with halogens. Unfortunately, the very crude mixtures obtained by direct extraction of urine with methylene chloride so contaminate the mass spectrometer ion source that only four or five samples can be run between source cleanings (37). It is possible to avoid this problem simply by reducing the polarity of the extraction solvent. When hexane is used to extract sulfuric acid hydrolysates of urines, the concentrated extracts can be examined with little more fouling of the source than in normal operation (3).

4. Field Desorption Mass Spectrometry

Field desorption mass spectra generally contain fewer peaks than even CI or NCI spectra. This feature makes FD particularly attractive for the analysis of complex mixtures. However, the erratic nature of FD mass spectra, especially FD ion abundances, are significant drawbacks to the use of the technique.

Sphon et al. have used FDMS to develop a screening technique for mycotoxins in foods (40). Figures 32A-5 and 32A-6 illustrate the spectra that they obtained from an extract of corn contaminated with both aflatoxins B1 and B2 and one of tomatoes that contained zearalenone. These spectra are reminiscent of the NCI spectrum of a human urine extract (Fig. 32A-4); it is clearly useful to know what you are looking for if you want to find specific components by these techniques. We can expect to see the application of FDMS to analysis of complex environmental mixtures to increase in the future.

D. TOXIC RESIDUES IDENTIFIED IN HUMANS AND THE FOOD CHAIN

Compared to the reports of MS investigations of pesticides and toxic substances (see Chapter 23) those that contain MS data concerning toxic materials in humans or the food chain are relatively few. The many reports of mass spectra of toxic compounds are an absolute prerequisite to applying MS to studies of environmental contamination. Another reason for the small number of reports is the difficulty that we have already noted in dealing with real samples from the environment. It is straightforward to obtain and interpret a mass spectrum for a single toxic substance. It is a qualitatively different problem to start with 10 or 100 g of human fat, separate toxic substances, and identify and quantify them by MS.

Table 32A-II lists the toxic substances that have been identified and/or quantified from human sources and from the food chain by MS techniques. Although the list is short compared to the EPA list of analytical reference standards (62) or

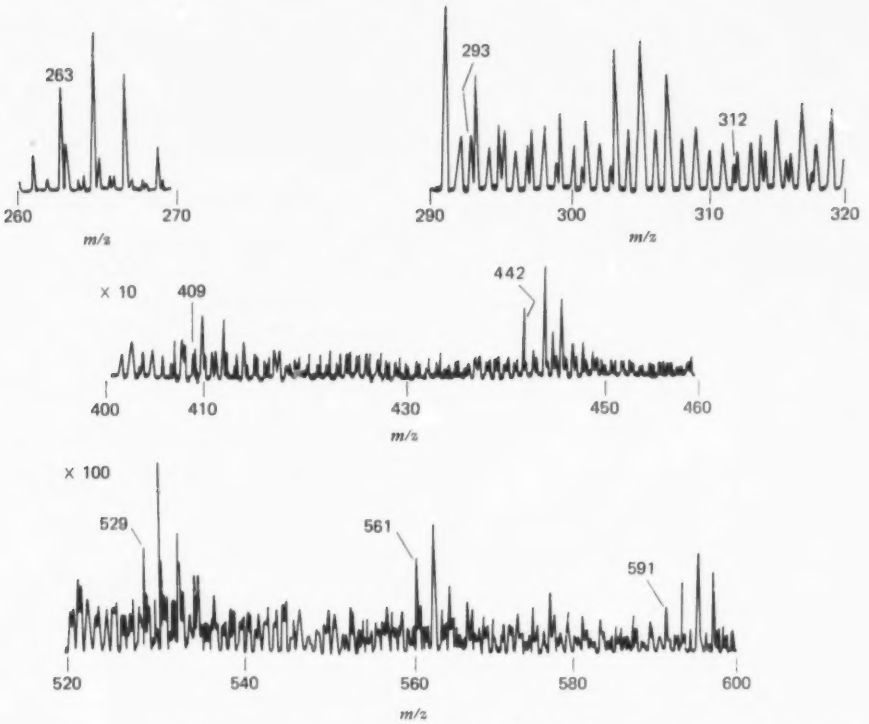


Figure 32A-4. Negative chemical ionization, 0.5 torr, methylene chloride extract of human urine adjusted to pH 6; HR was required to separate the polyhalide ions from the positive mass defect ions adjacent to them.

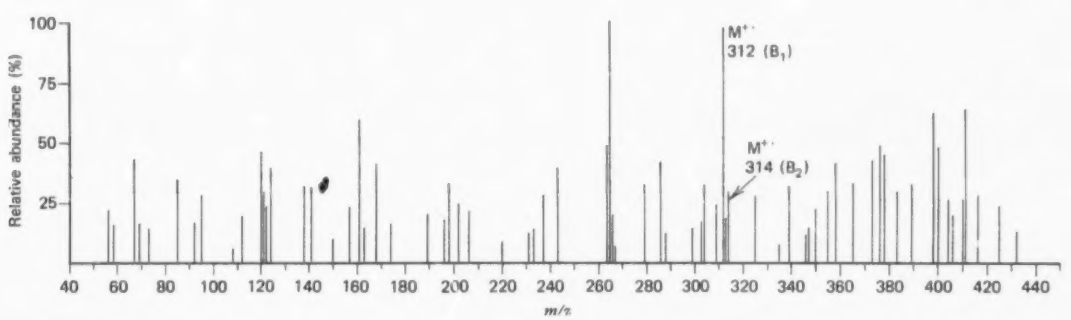


Figure 32A-5. Field desorption mass spectrum of an extract from corn containing ca. 15 ppb aflatoxin B_1 and 2.2 ppb aflatoxin B_2 (40).

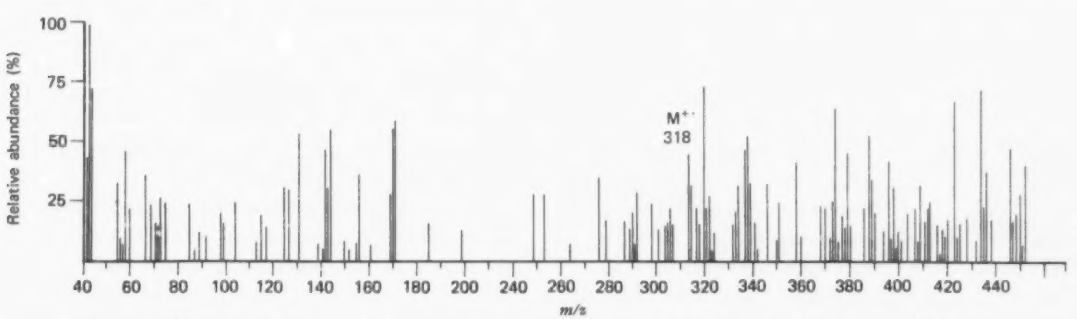


Figure 32A-6. Field desorption mass spectrum of zearalenone-contaminated tomatoes (40).

Table 32A-II Toxic Substances Shown Present by Mass Spectrometry

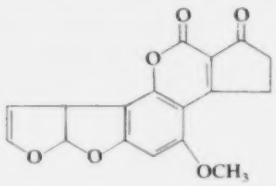
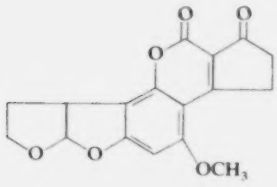
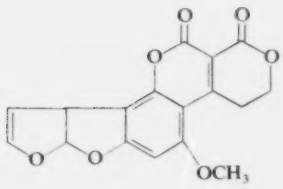
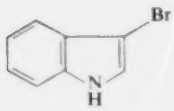
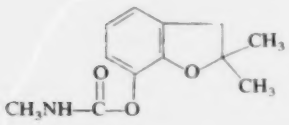
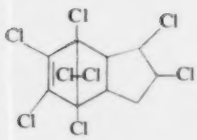
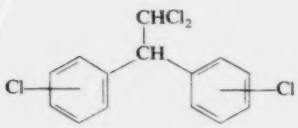
Name	Structure	Substrate	Concentration(s)	References
Aflatoxin B_1		Corn, cows' milk	~ 15 ppb	40, 60
Aflatoxin B_2		Corn	2.2 ppb	40
Aflatoxin G_1			N.D.[a]	59
3-Bromoindole		Fish	100–200 ppb	57a
Carbofuran		Carrots, celery, tomatoes, corn	0.02–1 ppm	41
Carbon tetrachloride	CCl_4	Human plasma	N.D.	61
Chlordane		Fish	<100 ppb	21, 27
DDD isomers		Human liver	2 ppm	17

Table 32A-II *(Continued)*

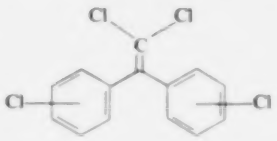
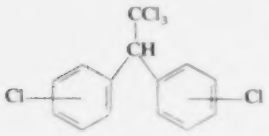
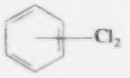
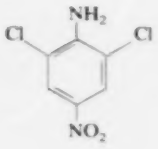
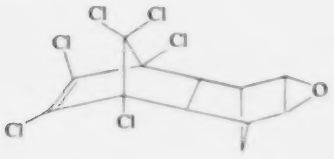
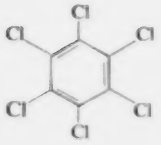
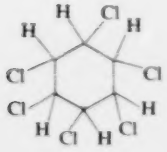
Name	Structure	Substrate	Concentration(s)	References
DDE isomers		Human adipose tissue, herring gull, bald eagle, human milk, fish, cheese	1–300 ppm	17 27 42 43 43 55
DDT isomers		Human adipose tissue, liver, milk, herring gull, fish	0.3–8 ppm	17 27 42 43
2,3-Dibromopropanol	$BrCH_2—CHBr—CH_2OH$	Human urine	<20 ppb	4
Dichlorobenzenes		Human plasma	N.D.	61
2,6-Dichloro-4-nitro-aniline		Peaches	0.01–1 ppm	25
Dieldrin		Human adipose tissue, milk, liver, and fish	0.004–1 ppm	17 21 27 43 57, 57a, 57b
HCB (hexachlorobenzene)		Fish, human kidney	0–62 ppm	27, 44 21, 23
HCH (1,2,3,4,5,6-hexachlorocyclo-hexanes, benzene hexa-chlorides, *sym*- BHC, Lindane)		Human liver, milk, frog eggs, cocoa beans	0.005–0.9 ppm	17 43 55

Table 32A-II *(Continued)*

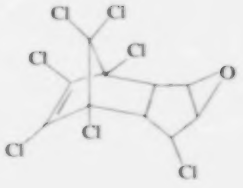
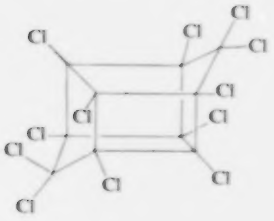
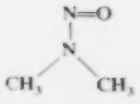
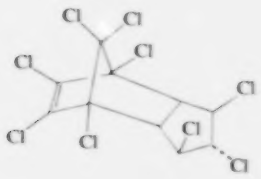
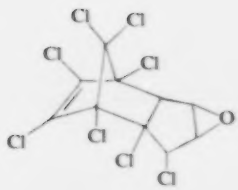
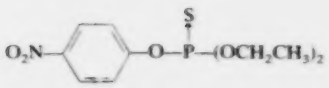
Name	Structure	Substrate	Concentration(s)	References
Heptachlorepoxide		Human adipose tissue, liver, and milk	0.004–0.5	17 43
Mirex		Human milk, herring gull, fish	0–210 ppm	2 42 43
N-Nitrosodimethylamine (DMN)		Human urine, cured meats, vegetables	1–115 ppb	28 45
N-Nitrosopyrrolidine		Human urine, cured meats	To 100 ppb	28 45
Nonachlor		Fish, human adipose tissue	< 100 ppb	27 53
Oxychlordane		Human milk	5 ppb	43
Parathion		Cherries	40–450 ppb	54

Table 32A-II (*Continued*)

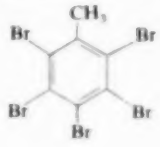
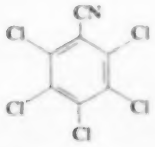
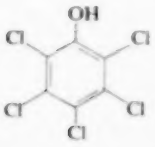
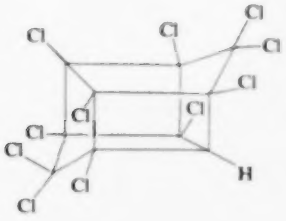
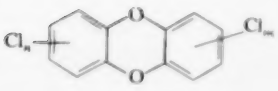
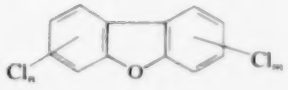
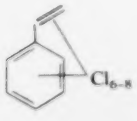
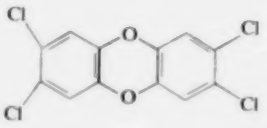
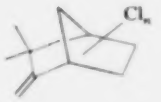
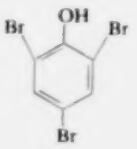
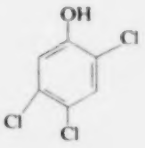
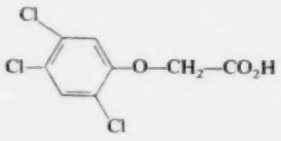
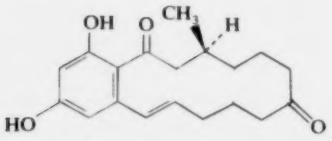
Name	Structure	Substrate	Concentration(s)	References
α-Pentabromotoluene		Grains, fish, sewage sludge	0.03–180 ppm	46
Pentachlorobenzonitrile		Carrots, celery	20 ppb	47
Pentachlorophenol		Human urine, seminal fluid, serum, grain, sugar products	0.01–120 ppm	3 48 49
Photomirex		Herring gull		42
Polychlorodibenzo-*p*-dioxins		Cattle, milk		50
Polychlorodibenzofurans		Rice oil, PCBs	4–5 ppm	51 52
Polychlorobiphenyls		Fish, human adipose tissue, human kidney, herring gull	to 5 ppm	23, 27 39, 42 56, 57, 57a, 57b
Polychlorostyrenes		Terns, fish, cormorant	N.D.	62, 63, 65

Table 32A-II (*Continued*)

TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin)		Fish, beef liver, soil, beef fat, human milk, beef milk	10–230 ppt	30 35 58
Tetrachloroethylene	$Cl_2C=CCl_2$	Human plasma	N.D.	61
Toxaphene		Fish	N.D.	27
2, 4, 6-Tribromophenol		Fish	100–200	57a
2, 4, 5-Trichlorophenol		Human urine	N.D.	3
2,4,5-Trichlorophenoxyacetic acid		Human urine, beef fat	10 ppb	3 49
Zearalenone		Tomatoes	N.D.	40

the National Cancer Institute collection of carcinogenic substances (63), it does contain most of the well-known persistent environmental contaminants. Phosphate and phosphothioate insecticides are not abundant in this list primarily because their lifetimes in the environment are relatively very short. The same is generally true of carbamate pesticides; only one example, carbofuran, appears in Table 32A-II.

The concentrations reported for toxic substances listed in Table 32A-II are generally in the range of parts per billion to parts per million. For compounds that are mutagenic and/or carcinogenic, as many of the compounds in Table

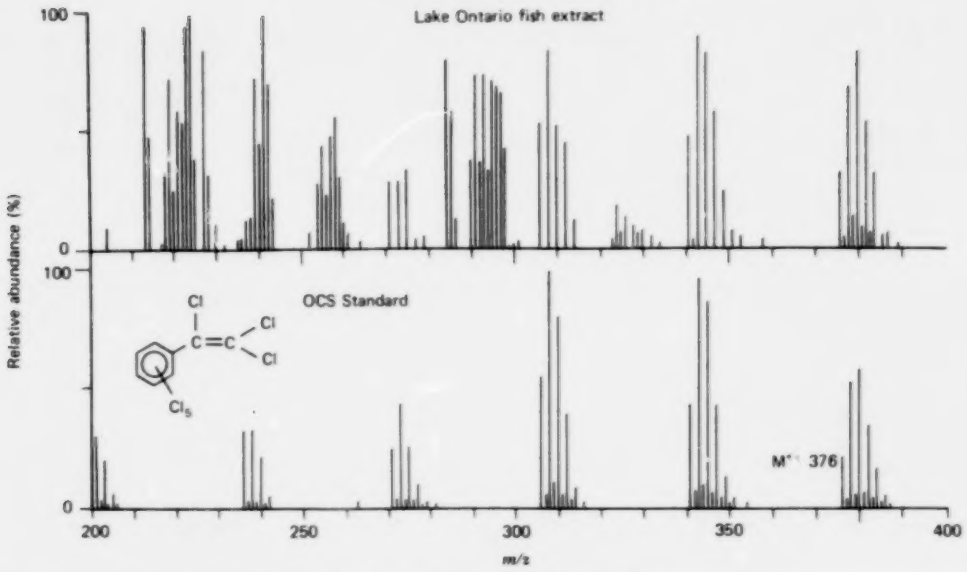


Figure 32-7. Electron impact MS of a fraction from a Lake Ontario fish extract and octachlorostyrene standard (64).

32A-II are, concentrations above the parts-per-billion level are definitely of significant environmental concern. The concentration of aflatoxin B_1 found in corn by Sphon et al. (40) is near the background level for aflatoxin B_1 in crops such as peanuts and corn. Since aflatoxin B_1 is extremely mutagenic and carcinogenic, its presence in the food chain at the part-per-billion level should help provide an answer to the general question: How clean is clean? One of the serious questions that will confront analytical toxicologists within the next decade is: What is the anticipated effect of contamination with a large number of toxic substances at the general level at which we find aflatoxins in the environment?

Not all the chemicals listed in Table 32A-II are pesticides or high-volume industrial chemicals. Octachlorostyrene was discovered and identified by GC/MS in samples of birds in Europe (62, 63) and in fish from the Great Lakes (64). Figure 32A-7 presents the EI spectrum of octachlorostyrene standard and that of a fraction from a Lake Ontario fish extract. Although other components are clearly present in this fraction, the MS correspondence along with comparisons of GC retention times allowed the conclusive identification of octachlorostyrene along with the hepta- and hexachlorostyrenes. One of the likely sources of octachlorostyrene in the environment is the electrolytic production of chlorine with carbon electrodes. Whatever the source, the amount of polychlorostyrenes in the environment must be significant if they appear as residues in bird and fish tissues.

E. CONCLUSIONS

The application of MS to studies of environmental analytical toxicology is one of the most rapidly growing fields in chemistry. Significant federal funding has dramatically increased the number of mass spectrometers that are dedicated to analysis of environment contamination. The ability of MS to detect unknown compounds, identify them, and determine their concentration has been clearly demonstrated for a substantial number of toxic materials. Its application to environmental analytical chemistry should provide the hard data that will be required for making appropriate regulatory decisions about environmental contamination. Fortunately, the history of science shows that when compounds are eliminated from the industrial economy, as DDT was, the total impact on the economy is generally quite small and the compounds are quickly replaced with equivalent or superior products.

We can anticipate new developments in cleanup techniques and in mass spectral approaches to extremely complex mixtures. The use of HRMS with NCI or FD should significantly aid compound

identification at the residue level. Further development of MIKES techniques for dealing with truly complex mixtures (64) should also be a significant step in the application of MS to environmental chemistry.

ACKNOWLEDGMENTS

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